



# Five Examples for NCBI BLAST

Teaching NCBI Resources  
Through Use Cases And Examples

## Introduction

BLAST programs from NCBI are powerful sequence alignment tools widely used in the analyses of biological sequences. In this booklet, we will work through five representative approaches that apply different functions provided by NCBI BLAST services ([blast.ncbi.nlm.nih.gov](http://blast.ncbi.nlm.nih.gov)) to address specific biological questions. Those examples will help you familiarize yourself with the web interface, better understand the capability of different BLAST programs, and learn the different result presentation formats plus their applications. With diverse sequence collections, you should be able to create your own working examples for use in your own teaching, or use different combinations of demonstrated capabilities to address your research needs.

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## Case 1: Identify Unknown Bacteria Using the 16S rRNA BLAST Database

### Goal

To validate the identity of an unknown bacterial sample using the 16S rRNA BLAST database

### Background

- Useful in microbiology lectures and laboratory courses
- About 5 minutes to complete the exercise
- A common exercise in microbiology is to identify a bacterial sample based on biochemical and growth properties. Another approach is to use targeted PCR amplification and analysis of genomic variations in the 16S rRNA gene to validate and even identify microbial samples. The 16S rRNA gene has a very conserved sequence overall that maintains its structure and role as a scaffold for the small subunit of the ribosome. This enables the use of “universal” primers for PCR amplification, but the gene contains a few regions of variability that allow it to be exploited for identification of microbial species.

### Steps

- From the BLAST home page ([blast.ncbi.nlm.nih.gov](http://blast.ncbi.nlm.nih.gov)), click **Nucleotide BLAST** to open the search page (A). You may want to use Reset Page (B) to return to default settings.
- Choose one or more sequences of a 16S rRNA region for an “unknown” bacterial sample. Retrieve those sequences from the FTP directory for this course ([bit.ly/2suC3lx](http://bit.ly/2suC3lx)). These sequences mimic the results of PCR amplification by a set of microbial universal primers.
- Paste one or more sequences into the **Enter Query Sequence** box (C), or upload the file through **Choose file** button (D).
- Select the **16S ribosomal RNA sequences** database using the database pull-down menu (E).
- Click the **BLAST** button to run the search.

Search results are shown on the next page.

**BLAST** » blastn suite

Home Recent Results Saved Strategies Help

Standard Nucleotide BLAST

blastn blastp blastx tblastn tblastx

BLASTN programs search nucleotide databases using a nucleotide query. [more...](#)

**Enter Query Sequence**

Enter accession number(s), gi(s), or FASTA sequence(s)

Or, upload file  No file chosen

Job Title

Enter a descriptive title for your BLAST search

☐ Align two or more sequences

**Choose Search Set**

Database ☐ Human genomic + transcript ☐ Mouse genomic + transcript ☒ Others (nr etc.): <http://bit.ly/2F2Ngml>

**16S ribosomal RNA sequences (Bacteria and Archaea)**

Organism Optional

Enter organism name or id—completions will be suggested  ☐ Exclude

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

☐ Sequences from type material

Limit to Optional

Entrez Query Optional

Enter an Entrez query to limit search

**Program Selection**

Optimize for

☒ Highly similar sequences (megablast)

☐ More dissimilar sequences (discontiguous megablast)

☐ Somewhat similar sequences (blastn)

Choose a BLAST algorithm

**BLAST**

Search database 16S ribosomal RNA sequences (Bacteria and Archaea) using Megablast (Optimize for highly similar sequences)

☐ Show results in a new window

**Nucleotide collection (nr/nt)**

**Genomic plus Transcript**

Human genomic plus transcript (Human G+T)

Mouse genomic plus transcript (Mouse G+T)

**Other Databases**

Nucleotide collection (nr/nt)

**16S ribosomal RNA sequences (Bacteria and Archaea)**

Reference RNA sequences (refseq\_ma)

RefSeq Representative genomes (refseq\_representative\_genomes)

RefSeq Genome Database (refseq\_genomes)

Whole-genome shotgun contigs (wgs)

Expressed sequence tags (est)

Sequence Read Archive (SRA)

Transcriptome Shotgun Assembly (TSA)

High throughput genomic sequences (HTGS)

Patent sequences (pat)

Protein Data Bank (pdb)

Reference genomic sequences (refseq\_genomic)

Human RefSeqGene sequences (RefSeq\_Gene)

Genomic survey sequences (gss)

Sequence tagged sites (dbsts)

## Case 1 (cont.)

### Interpretation

- It is common for this conserved region to match many bacterial sequences. However, by identifying those closest to the query sequence, it is possible to establish a likely identity for the unknown sample.
- The **Descriptions** section of the BLAST report provides a quick view of the results (A). For very similar results like these, Max score is often, but not always, the important statistic. Consider the percent identity and query coverage, and confirm identification by looking in the **Alignments** section (B).
- You can use the **Formatting options** menu near the top of the results page to more easily compare the alignments (such as) by changing the **Alignment View** to one of the **...with dots for identities** formats (no illustrated).
- Finally, the **Distance tree of results** link (C) provides a quick, BLAST-based phylogenetic tree of the alignments. This is another way to identify the sequence that is most similar to the unknown sample.

**BLAST** » blastn suite » RID-894HX93S014 [Home](#) [Recent Results](#) [Saved Strategies](#) [Help](#)

**BLAST Results**

[Edit and Resubmit](#) [Save Search Strategies](#) [Formatting options](#) [Download](#) [YouTube](#) [How to read this page](#) [Blast report description](#)

**Job title: 7 sequences (Shigella dysenteriae)**

Results for: 1:IdlQuery\_141321 Shigella dysenteriae(1487bp)

RID: 8965H3M1014 (Expires on 02-16 03:14 am)

Query ID: IdlQuery\_51349  
Description: unknown Shigella  
Molecule type: nucleic acid  
Query Length: 1487

Database Name: rRNA\_tpestrains/prokaryotic\_16S\_ribosomal\_RNA  
Description: 16S ribosomal RNA (Bacteria and Archaea)  
Program: BLASTN 2.8.0+ [Citation](#)

Other reports: [Search Summary](#) [Taxonomy reports](#) [Distance tree of results](#)

[Graphic Summary](#)  
[Descriptions](#)

Sequences producing significant alignments:

Select: [All](#) [None](#) Selected: 0

[Alignments](#) [Download](#) [GenBank](#) [Graphics](#) [Distance tree of results](#)

	Description	Max score	Total score	Query cover	E value	Ident	Accession
<input type="checkbox"/>	Shigella dysenteriae strain ATCC 13313 16S ribosomal RNA gene, partial sequence	2747	2747	100%	0.0	100%	NR_026332.1
<input type="checkbox"/>	Shigella flexneri strain ATCC 29903 16S ribosomal RNA gene, partial sequence	2669	2669	100%	0.0	99%	NR_026331.1
<input type="checkbox"/>	Escherichia fergusonii strain ATCC 35469 16S ribosomal RNA, complete sequence	2658	2658	100%	0.0	99%	NR_074902.1
<input type="checkbox"/>	Escherichia marmotae strain HT073016 16S ribosomal RNA, partial sequence	2652	2652	100%	0.0	99%	NR_136472.1
<input type="checkbox"/>	Citrobacter amalonaticus strain CECT 863 16S ribosomal RNA gene, partial sequence	2542	2542	100%	0.0	98%	NR_104823.1
<input type="checkbox"/>	Kosakonia sacchari strain SP1 16S ribosomal RNA, partial sequence	2529	2529	100%	0.0	97%	NR_118333.1
<input type="checkbox"/>	Enterobacter massiliensis strain JC163 16S ribosomal RNA gene, partial sequence	2525	2525	100%	0.0	97%	NR_125600.1
<input type="checkbox"/>	Salmonella enterica subsp. enterica strain Ty2 16S ribosomal RNA, partial sequence	2519	2519	100%	0.0	97%	NR_074799.1
<input type="checkbox"/>	Salmonella enterica subsp. arizonae strain ATCC 13314 16S ribosomal RNA gene, partial sequence	2519	2519	100%	0.0	97%	NR_041696.1
<input type="checkbox"/>	Salmonella enterica subsp. enterica strain LT2 16S ribosomal RNA, partial sequence	2514	2514	100%	0.0	97%	NR_074910.1

[Download](#) [GenBank](#) [Graphics](#) [Next](#)

Shigella dysenteriae strain ATCC 13313 16S ribosomal RNA gene, partial sequence  
Sequence ID: NR\_026332.1 Length: 1487 Number of Matches: 1

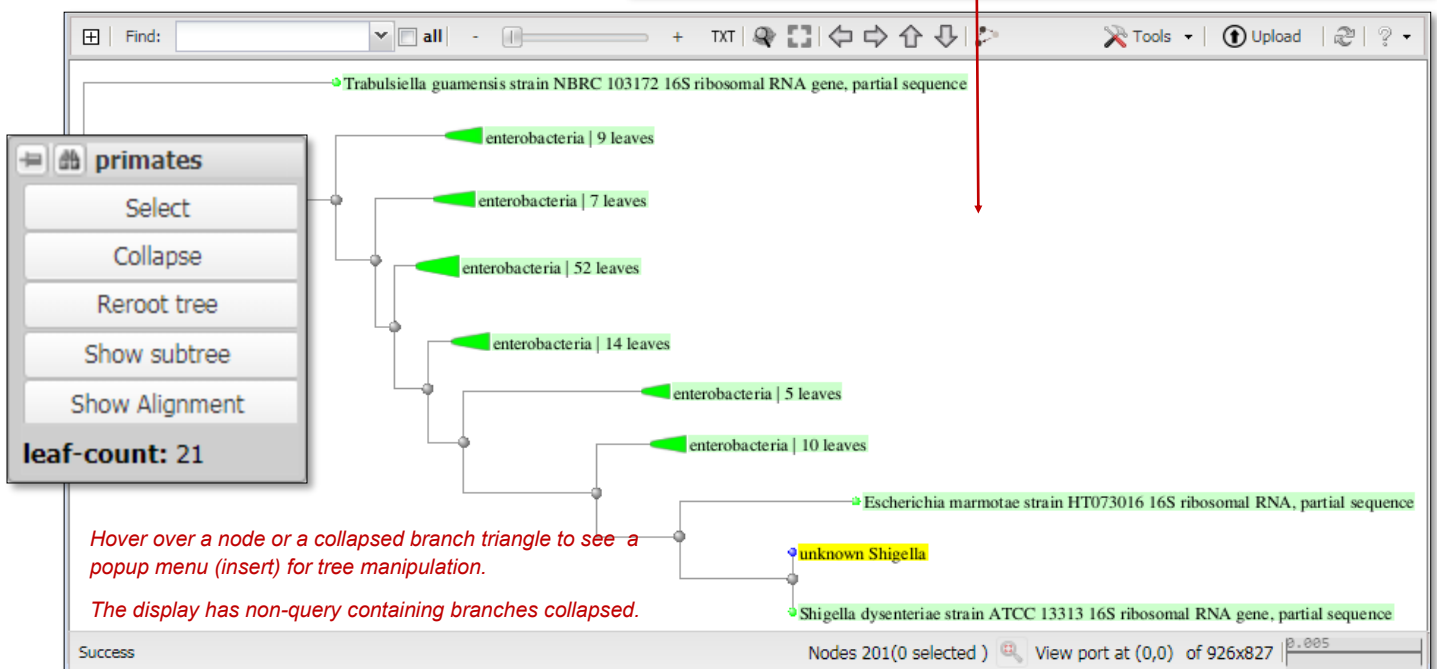
Range 1: 1 to 1487 [GenBank](#) [Graphics](#)

Score	Expect	Identities	Gaps	Strand
2747 bits(1487)	0.0	1487/1487(100%)	0/1487(0%)	Plus/Plus

Query 1 TGGCTCAGATTGAACGCTGGCGGCAAGCCTAACACATGCAAGTCGAACGGTAACAGAAAG 60  
Sbjct 1 TGGCTCAGATTGAACGCTGGCGGCAAGCCTAACACATGCAAGTCGAACGGTAACAGAAAG 60

Query 61 CAGCTTGCTGTTTGTCTGACGAGTGGCGGACGGGTGAGTAATGCTGGGAAACTGCCCTGAT 120  
Sbjct 61 CAGCTTGCTGTTTGTCTGACGAGTGGCGGACGGGTGAGTAATGCTGGGAAACTGCCCTGAT 120

Query 121 GGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAGAG 180  
Sbjct 121 GGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAGAG 180



## Case 2: Identify PCR Primer Pairs for Amplifying the Coding Region of an mRNA

### Goal

Use Primer-BLAST to find a PCR primer pair that can be used in the laboratory to amplify a coding region of an mRNA

### Background

- Useful in molecular biology and biochemistry courses
- About 10 minutes to complete the exercise

A common laboratory exercise in molecular biology and biochemistry courses is to design PCR primers for a target sequence. This target sequence is often a protein coding sequence that can be subsequently ligated into an expression plasmid and then used for other lab sessions, such as expression and characterization of the protein, mutagenesis, or promoter analysis.

### Steps

- Retrieve [NM\\_000250](#), the RefSeq mRNA sequence for Human Myeloperoxidase, MPO (**A**) from the Nucleotide database
- Use the web browser's **Find in page** function (ctrl+F) to find the **CDS** feature. The coding sequence (CDS) for this gene starts at position 178 and ends at position 2415. It is 2238 nucleotides long (**B**). Write down these positions because you'll need them in subsequent steps.
- On the right side of the record under **Analyze this sequence**, click **Pick Primers** (**C**) to open the Primer-BLAST page with the accession already entered as the template.
- To amplify the CDS region, set the ranges for **Forward** and **Reverse primers** outside of the CDS positions in the record. For forward primer range set the range from 138 to 178. For the reverse primer, set the range from 2415 to 2500 (**D**). Also, adjust the **PCR product size** by increasing the **Max** size to 2500 (**E**), so that the entire CDS amplifies.
- Click **Get Primers** to run the search (not illustrated), which uses the default database RefSeq mRNA database limited to Human.

**Homo sapiens myeloperoxidase (MPO), mRNA** **A**

NCBI Reference Sequence: NM\_000250.1

[FASTA](#) [Graphics](#)

Go to:

LOCUS NM\_000250 3215 bp mRNA linear PRI 22-JAN-2018

DEFINITION Homo sapiens myeloperoxidase (MPO), mRNA.

ACCESSION NM\_000250

VERSION NM\_000250.1

FEATURES

Location/Qualifiers
source 1..3215
/organism="Homo sapiens"
/mol_type="mRNA"
/db_xref="taxon:9606"
/chromosome="17"
/map="17q22"
gene 1..3215
/gene="MPO"
/note="myeloperoxidase"
/db_xref="GeneID:4353"
/db_xref="HGNC:HGNC:7218"
/db_xref="MIM:606989"
exon 1..331
/gene="MPO"
/inference="alignment:Splign:2.0.8"
misc_feature 127..129
/gene="MPO"
/note="upstream in-frame stop codon"
<b>CDS 178..2415</b>
/gene="MPO"
/EC_number="1.11.2.2"
/codon_start=1
/product="myeloperoxidase precursor"
/protein_id="NP_000241.1"

**B**

**CDS 178..2415 /gene="MPO"**

**Range**

	From	To
Forward primer	138	178
Reverse primer	2415	2500

**D**

**PCR Template**

Enter accession, gi, or FASTA sequence (A refseq record is preferred)  [Clear](#)

NM\_000250.1

Or, upload FASTA file  No file chosen

**Primer Parameters**

Use my own forward primer (5'→3' on plus strand)  [Clear](#)

Use my own reverse primer (5'→3' on minus strand)  [Clear](#)

PCR product size

Min	Max
70	2500 <b>E</b>

# of primers to return

Primer melting temperatures (T<sub>m</sub>)

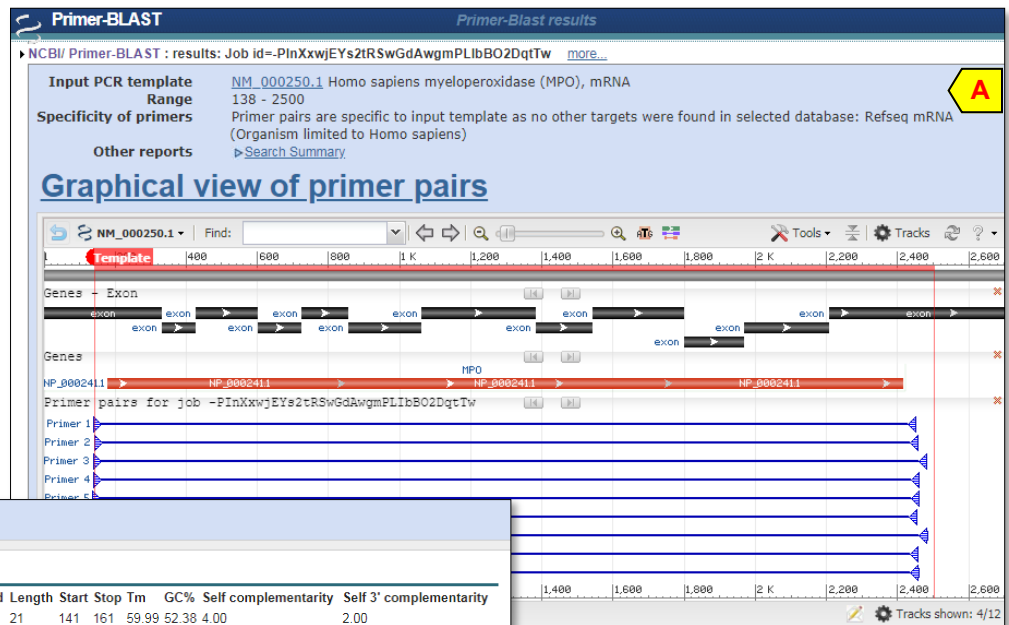
Min	Opt	Max	Max T <sub>m</sub> difference
57.0	60.0	63.0	3



## Case 2 (cont.)

### Interpretation

- It is important to find PCR Primer pairs that will amplify only the sequence intended. In this case, the selected PCR Primer pairs should amplify only the Human MPO transcript.
- The top of the results page (A) summarizes the Primer BLAST search. The **Detailed primer reports** section (B) lists a set of primer pairs and their key characteristics for you to select from.
- You can find suggestions on how to get primers specific to your template from the tips page, at [www.ncbi.nlm.nih.gov/tools/primer-blast/search\\_tips.html](http://www.ncbi.nlm.nih.gov/tools/primer-blast/search_tips.html)
- It is most important for both primers in a pair to have similar Tm values and GC percentages when possible. In addition, self-complementarity should be low to prevent primers binding to themselves and each other, rather than the template.
- By default, Primer BLAST uses stringent parameters. You can relax them if you are not able to find any suitable primers. Be aware that this may increase the potential of misprimed amplification due to annealing to secondary annealing sites on other templates.



### Detailed primer reports

**Primer pair 1**

Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	Plus	21	141	161	59.99	52.38	4.00	2.00
Reverse primer	Minus	21	2451	2431	59.58	52.38	5.00	3.00

Product length 2311

**Products on intended target**  
 >NM\_000250.1 Homo sapiens myeloperoxidase (MPO), mRNA

product length = 2311  
 Forward primer 1 GGTACAAAGGGGATTGAGCA 21  
 Template 141 ..... 161  
 Reverse primer 1 ATATACCCCTCACTGCTGCAC 21  
 Template 2451 ..... 2431

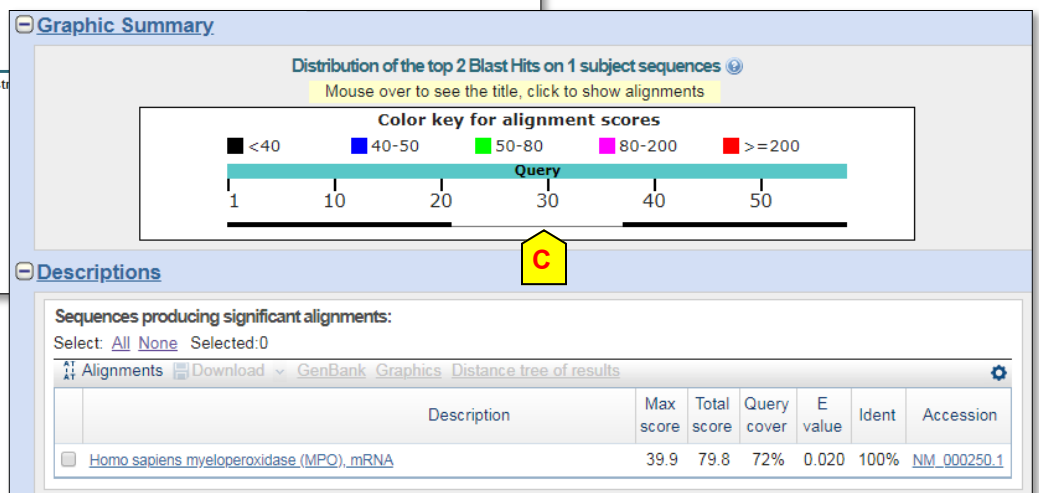
**Primer pair 2**

Sequence (5'→3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	Plus	21	143	163	59.99	52.38	4.00	2.00
Reverse primer	Minus	21	2457	2437	59.58	52.38	5.00	3.00

Product length 2315

**Products on intended target**  
 >NM\_000250.1 Homo sapiens myeloperoxidase (MPO), mRNA

product length = 2315  
 Forward primer 1 TACAAAGGGGATTGAGCAGC 21  
 Template 143 ..... 163  
 Reverse primer 1 GCCCAGATATACCCCTCACTG 21  
 Template 2457 ..... 2437



To confirm the specificity, you can run a Nucleotide BLAST search, with program set to **blastn**, against the human RefSeq RNA dataset. To force BLAST to align each primer independently in a single search, while preserving their spatial relationship, make sure you provide the primer set in this example format, GGTACAAAGGGGGATTGAG-CANNNNNNNNNNNNNNNNNNNNATATACCCCTCACTGCTGCAC. In the result display, the primers matching to different parts of the same target sequence have a thin line connecting them (C).

## Case 3: Generate Species and Gene Phylogenetic Trees

### Goal

Use blastn and blastp to find homologous molecules and generate distance trees.

### Background

- Useful in general biology, molecular biology, and vertebrate zoology courses
- About 15 min for each of two examples
- Example i) generates a phylogeny of apes using complete mitochondrial genome sequences. Example ii) builds a gene (protein) tree for the creatine kinases, a small protein family with four or more members in vertebrate proteomes.

### i) Ape Phylogeny

#### Steps:

- Retrieve the ring-tailed lemur mitochondrial genome sequence, accession number [NC\\_004025.1](#), from the Nucleotide database. You can use this sequence as a query to retrieve and align the ape mitochondrial genomes using blastn.
- Click **Run BLAST** (A) on that Nucleotide page to load the blast search form.
- Select the **RefSeq Genomic sequences (refseq\_genomic)** as the database (B). This database contains all genomic sequences from NCBI's RefSeq project. The information icon ? links to a detailed description of the database.
- Paste in the following list of accessions for apes mitochondrial genomes to the **Entrez Query** box (C):  
 NC\_001643 OR NC\_001644 OR NC\_001645 OR NC\_001646 OR NC\_002082 OR NC\_002083 OR NC\_011120 OR NC\_011137 OR NC\_012920 OR NC\_013993 OR NC\_014042 OR NC\_014045 OR NC\_014047 OR NC\_014051 OR NC\_018753 OR NC\_021957 OR NC\_023100 OR NC\_033882 OR NC\_033883 OR NC\_033884 OR NC\_033885
- Adjust the BLAST program to **More dissimilar sequences** (D), expand the **Algorithm parameters** section and set the **Expect** threshold to 1e-46 (E). A page with the above setting is at <http://bit.ly/2qBBJo4>
- Click **BLAST** button to submit the search.
- The results showed nearly full-length matches to the lemur query. These include mitochondrial genome sequences from gorillas, chimpanzees, orangutans, gibbons, and four distinct taxa in the genus Homo – modern humans, plus three extinct groups: the *Neanderthal* and *Denisovan* hominids as well as *Homo heidelbergensis* (not shown). The Taxonomy report (F) shows the taxa represented in the output.
- Click the **"Distance tree of results"** link to generate a tree.

**Lemur catta mitochondrion, complete genome**

NCBI Reference Sequence: NC\_004025.1

[FASTA](#) [Graphics](#)

Go to: ☒

LOCUS	NC_004025	17036 bp	DNA	circular	PRI	01-FEB-2010
DEFINITION	Lemur catta mitochondrion, complete genome.					
ACCESSION	NC_004025					
VERSION	NC_004025.1					

**Analyze this sequence**

**Run BLAST**

Pick Primers

Highlight Sequence Features

Find in this Sequence

**Choose Search Set**

Database: ☐ Human genomic + transcript ☐ Mouse genomic + transcript ☒ Others (nr etc.): **B**

Reference genomic sequences (refseq\_genomic)

Organism:  Enter organism name or id—completions will be suggested ☐ Exclude  Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown

Exclude: ☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Limit to: ☐ Sequences from type material

Entrez Query:  NC\_001643.1 OR NC\_001644.1 OR NC\_001645.1 OR NC\_001646.1 OR NC\_002082.1 OR NC\_002083.1 OR NC\_011120.1 OR NC\_011137.1 OR NC\_012920.1 OR NC\_013993.1 OR NC\_014042.1 OR NC\_014045.1 OR NC\_014047.1 OR NC\_014051.1 OR NC\_018753.1 OR NC\_021957.1 OR NC\_023100.1 OR NC\_033882.1 OR NC\_033883.1 OR NC\_033884.1 OR NC\_033885.1 **C**

**Program Selection**

Optimize for: ☐ Highly similar sequences (megablast) ☒ More dissimilar sequences (discontiguous megablast) **D** ☐ Somewhat similar sequences (blastn)

Choose a BLAST algorithm

**BLAST** Search database Reference genomic sequences (refseq\_genomic) using Discontiguous megablast

☐ Show results in a new window

**Algorithm parameters** Note: Parameter values that differ from the default are highlighted in yellow and

**General Parameters**

Max target sequences:  100 Select the maximum number of aligned sequences to display

Short queries: ☒ Automatically adjust parameters for short input sequences

Expect threshold:  1e-46 **E**

**Description** NCBI Genomic Reference Sequences

**Program** BLASTN 2.8.0+ [Citation](#)

Your search is limited to records that include: entrez query: 1

[Edit and Resubmit](#) [Save Search Strategies](#) [Formatting options](#)

**Job title:** NC\_004025:Lemur catta mitochondrion, complete genome

**RID** DAFNAZPP014 (Expires on 04-18 08:24 am)

**Query ID** NC\_004025.1

**Description** Lemur catta mitochondrion, complete genome

**Molecule type** dna

**Query Length** 17036

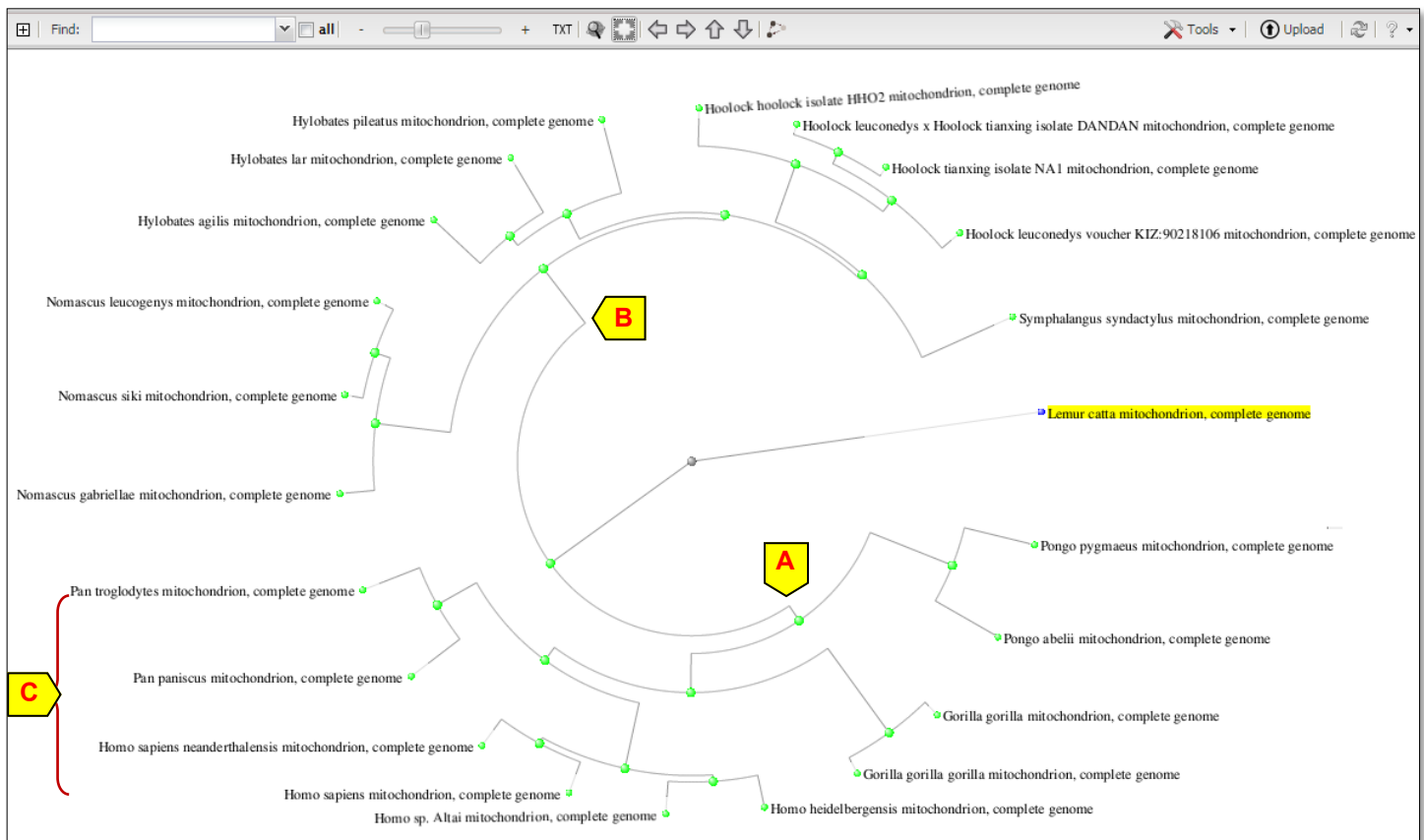
**Other reports:** [Search Summary](#) [Taxonomy reports](#) [Distance tree of results](#) [MSA viewer](#)

**F**

## Case 3 (cont.)

### Interpretation:

- The tree supports the two distinct groups of apes: the Great apes (*Hominidae*, **A**) containing humans, chimpanzees, gorillas and orangutans, and gibbons (*Hylobatidae*, **B**). It also shows the chimpanzee (*Pan troglodytes*) and the bonobo (*Pan paniscus*) as the closest living relatives of humans and the Neanderthal as the closest extinct relative (**C**).
- Note that this tree is based completely on blastn's local and pairwise comparisons to the query (lemur) sequence. It produces a reasonable alignment for generating the tree due to overall conservation in the mitochondrion genomes for this group of organisms. The most accurate tree, however, requires a true multiple sequence alignment (using a tool such as **MUSCLE**) for nucleotide sequences. NCBI does not have a separate nucleotide multiple alignment tool. Example ii) below uses a true protein multiple alignment through COBALT to generate a protein tree.



### ii) Creatine Kinase protein tree

#### Steps

- Retrieve human creatine kinase B-type protein, [NP\\_001814.2](#), from the Protein database. Use it as a blastp query to retrieve the tetrapod vertebrate creatine kinases, then perform a multiple sequence alignment for the matched sequences using COBALT to make a protein tree.
- Click **Run BLAST** on that Protein page to load the blastp search form (as described in Case 3i).
- Change the database to **Reference proteins**, which contains NCBI RefSeqs used in or generated by the NCBI genome annotation pipelines. Click the information icon **?** to see more information.
- Type **tetrapods** in the Organism box and select **taxid:32523** from the list. This limits the search to sequences from this group of organisms.
- Eliminate predicted entries by checking the **Exclude** box for **Models** to get a smaller tree.
- Expand the **Algorithm parameters** section and set the Expect threshold to **1e-55** to further limit hits returned by BLAST. Click the **BLAST** button to run the search.



## Case 3 (cont.)

### ii) Creatine Kinase Protein Tree

#### Steps

- In the results page, click **Multiple alignment** (A) to send matched proteins to COBALT for true multiple sequence alignment. Note that the multiple alignment will involve residues that were not present in the local BLAST alignments, such as the signal peptide from the mitochondrial targeted proteins.

**BLAST Results**

Your search is limited to records that include: tetrapods (taxid:32523) ; and exclude: models (XM/XP) ▶ [Full Entrez Query](#)

[Edit and Resubmit](#) [Save Search Strategies](#) [Formatting options](#) [Download](#) [YouTube](#) [How to read this page](#) [Blast report](#)

**Job title: (2) - NP\_001814:creatine kinase B-type [Homo sapiens]**

RID [8MWR11F5014](#) (Expires on 02-20 13:46 pm)

Query ID	<a href="#">NP_001814.2</a>	Database Name	refseq_protein
Description	creatine kinase B-type [Homo sapiens]	Description	NCBI Protein Reference Sequences
Molecule type	amino acid	Program	BLASTP 2.8.0+ ▶ <a href="#">Citation</a>
Query Length	381		

Other reports: [Search Summary](#) [Taxonomy reports](#) [Distance tree of results](#) [Multiple alignment](#) [MSA viewer](#)

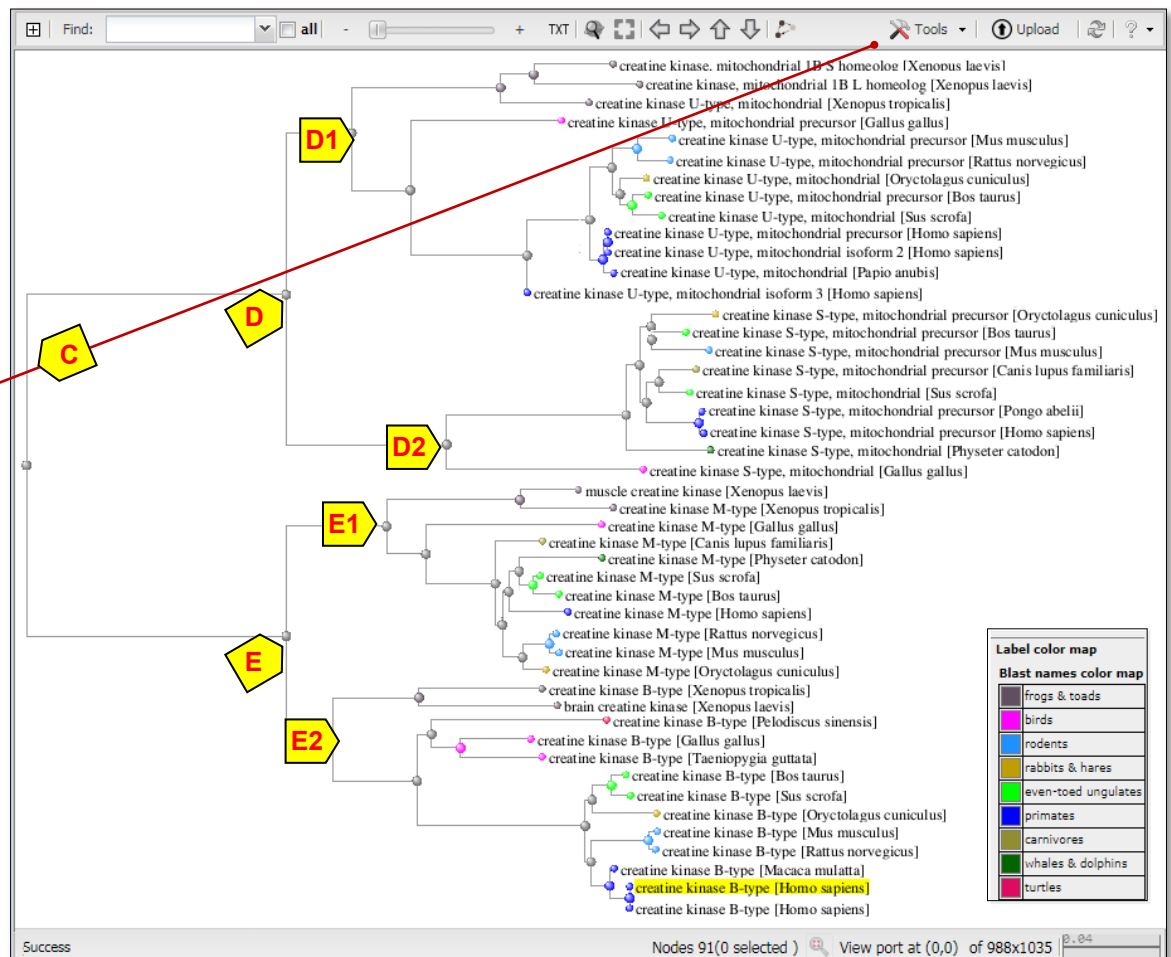
**COBALT** **Constraint-based Multiple Alignment** [Recent Results](#) [Help](#)

[Phylogenetic Tree](#) [Edit and Resubmit](#) [Back to Blast Results](#) [Download](#)

**Multiple Alignment Results - (2) - NP\_001814:creatine kinase B-type [Homo sapiens] - Cobalt RID 8MWTGY4C211 (46 seqs)**

**Tools**

- Download ▶
- Layout ▶
- Sort ▶
- Zoom behavior ▶
- Clear selection
- Clear subtree
- Clear rerooting
- Expand all**
- Edit labels



- From the COBALT results, click the **Phylogenetic Tree** (B) at the top to generate the protein tree.
- Toggle the **Collapse Mode** to **Show all** to expand all leaf nodes (C), if some leaf nodes are not expanded.

#### Interpretation

- The resulting tree (right) is complicated by the presence of multiple isoforms from the same gene in a particular species. However, there are clearly two distinct groups of proteins, mitochondrial (D) and cytoplasmic (E), with two types of genes in each, U-type (D1) and S-type (D2) for mitochondrial group, and M-type (E1) and B-Type (E2) for Cytoplasmic group. This is a good example of a gene (protein) tree as compared to a species tree. Notice that mouse and human have proteins in all four groups, and that the mouse M-type is more similar to the human M-type than it is to the mouse U-type. Within a particular protein type though, tetrapod relationships are about as expected. For instance, the mouse M-type is closer to the rat M-type than either is to the human M-type.

## Case 4: Annotate a Metagenomic Contig

### Goal:

Use blastx to find potential proteins/genes on a genomic contig.

### Background

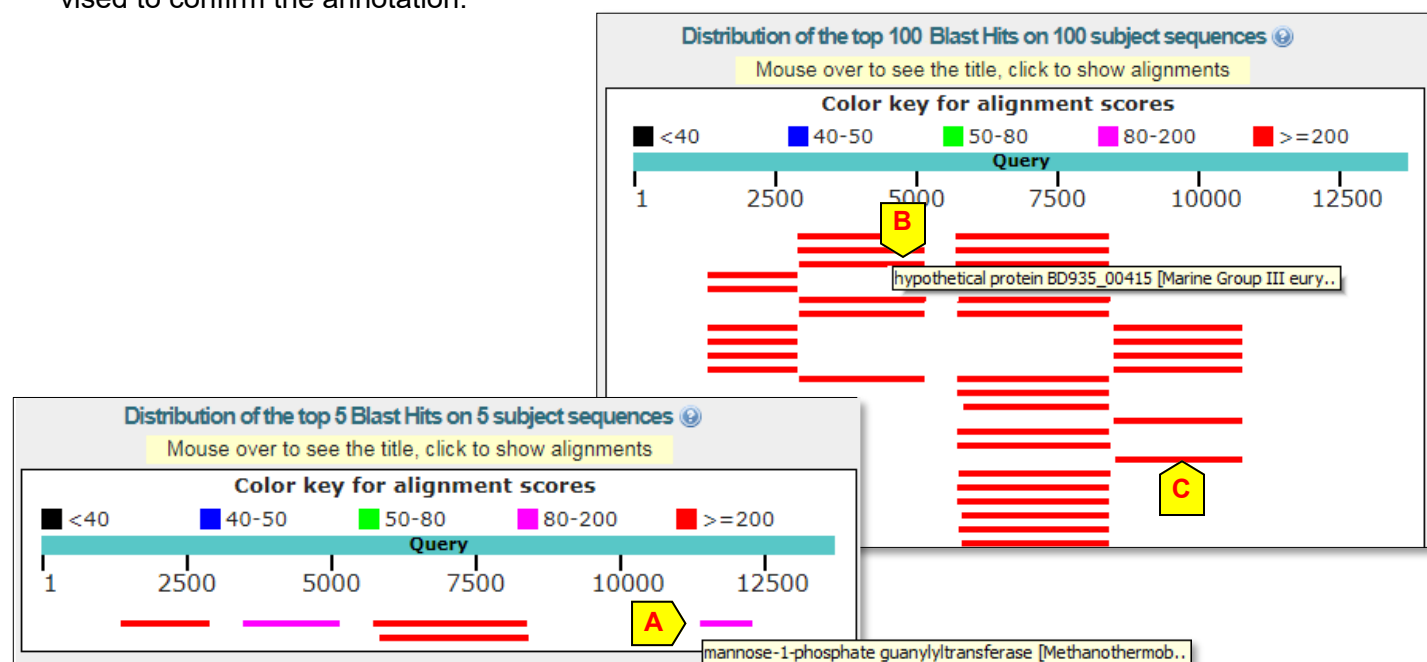
- Useful in molecular biology and microbiology courses
- About 10 min to complete the exercise
- Other software is often used for large-scale gene prediction and annotation, but blastx nicely illustrates the principles.

### Steps

- Retrieve accession number [MIZB01000007.1](#) from the Nucleotide database. This is a 13.7 kb genomic contig assembled from an Euryarchaeal marine metagenomic reads.
- Click **Run BLAST** on that Nucleotide page, click the **blastx** tab at the top to change the program.
- Select the **Model Organisms (landmark)** database. The reason for selecting this database is that it is small and non-redundant, with proteomes from a wide taxonomic group, thus your search will be quick and the result will be more concise. The information icon ? provides more information. **Tips:** For a larger database, such as *nr*, we suggest setting the **Expect** threshold to 1e-6 (10 to the minus 6th power) or lower, but that is usually not necessary with the *landmark* database.
- Use the **Organism** field to limit the search to **archaea (taxid:2157)** to match the organism source of the query, and creates a cleaner set of results. However, choose such limits carefully to match the goals you want to achieve. Click the **BLAST** button to submit the search.
- You may want to save the Request ID (RID) number found on the results page for later use, although they are saved in **Recent Results** page for the current browser session. All RIDs expire after about 36 hours.
- We want to compare results with a search against the protein *nr* database. Click the **Edit and resubmit** link near the top of the page, then change the database to *nr*. Set the **Expect** threshold to 1e-6, check that the Organism limit remains, then click the **BLAST** button.

### Interpretation

- The search against the Landmark database (**A**) suggests four possible genes on the contig, including: Hef nuclease, DNA topoisomerases, alanine-tRNA ligase, and mannose-1-phosphate guanylyltransferase.
- The value of searching multiple databases: the search against Landmark identifies the Hef nuclease, which was only labeled “hypothetical protein” in the search against *nr* (**B**). The search against *nr* adds the oligopeptide transporter, OPT family (**C**).
- Further analyses, including close examination of conserved domains in the proposed proteins, are advised to confirm the annotation.



## Case 5: Examine Conserved Domains and Solved Structures to Support a Protein Annotation

### Goals:

- Use blastx to find a structure record related by sequence to your annotated protein
- Confirm that your protein contains important sequence motifs for the conserved domain
- View these motifs on the solved structure using the iCn3D or Cn3D viewers.

## Background:

- Useful in many biology courses.
- About 15 min

## Steps

- Run a blastx search with the contig from Case 4, [MIZB01000007.1](#) as the query.
- Choose **Protein Data Bank proteins (pdb)** as the database, which contains solved structures from NCBI's Structure database. Lower the **Expect** threshold to 1e-6 to return only the better alignments.
- On the results page, we'll focus on the best hit, an alanyl-tRNA synthetase (another name for alanine-tRNA ligase). In the Descriptions section of the results page, click the Accession link for [3WQY\\_A](#), the crystal structure of Archaeoglobus fulgidus alanyl-trna synthetase in complex with wild-type tRNA(ala).
- In the Protein record for 3WQY\_A, click **Identify Conserved Domains** under **Analyze this Sequence**.
- Notice the match to AlaRS\_core domain, when viewed in **Full Results** mode (A).
- To see how residues in the motifs match the sequences used to construct this domain, click on the AlaRS\_core bar (B) in the "Specific hits" row to open the domain.
- In the **Conserved Features/Sites** tab, click on **motif 1** and **Scroll to Sequence Alignment Display** (C), the 3WQY\_A sequence is called the **query** and motif 1 is marked by the # symbols.

**Conserved domains on** [gi|1101737691|gb|OIR21235.1|]

alanine--tRNA ligase [Marine Group III euryarchaeote CG-Epi1]

**Protein Classification**

alanine--tRNA ligase (domain architecture ID 11486935)

alanine--tRNA ligase catalyzes the attachment of alanine to the 3' OH group of ribose of tRNA(Ala)

**Graphical summary** ☐ Zoom to residue level [show extra options >](#)

Query seq.

Specific hits

alaS

AlaRS\_core

Sequence Alignment include consensus sequence ?

Reformat Format: Hypertext Row Display: up to 10 Color Bits: 2.0 bit Type Selection: the most similar members

Feature 2

gi 6226166 6 TEEVRSKFITYFKAn---NHTHPASSLi--pDM

query 58 LDEAREAFRLRFEEKn---KHTRVDRASVvarwR

gi 22096190 60 ISEMREYYLSFFEAR---GHTRLD RYPVvarwR

gi 22096191 61 VWEAGEEFFRFFERh---DHEVLD RYPVvarwR

gi 6707751 59 LDEMREKFLRFFEKheiyPHGRVK RYPVprwR

gi 14286171 62 VKEAREKFLSFFEKr---GHTRIPPKPVLarwRED1--YLTIASIVDFQPHVtSGLVPPPanPLIVSQPSIRLedidnvg 150

gi 6707749 46 VGEAREAFSLFFEKH---GHTRVPPRPVvarwRED1--YLTIASIVVFQPHVtSGLVPPPanPLVISQPSIRLedidnvg 120

gi 3334348 62 LDEMREYYLNFFERR---GHGRIERYPVvarwRTD1--YLTIASIADFQPHVtSGVAPPanPLTISQPCIRLddldsvg 136

gi 22096203 62 LSEMRDAFIKFFEKr---GHKFLKPPYPVprwREDv--LLVNASIYDFQPHVtSGIVRPPgnPIVMSQPSIRMndvdldvg 136

gi 2507425 5 TAEIRQAFLDFFHsk---GHQVVASLsv--pHNDptlLFTNAGMNQFKDVF-LGLDKRnysRATTSQRCVRAggkhdn1 78

Conserved Features/Sites ? PubMed References ?

active site motif 1 motif 2 motif 3

Feature 2: motif 1

Evidence:

- Comment: characteristic motif of class II is part of dimer interface

Scroll to Sequence Alignment Display

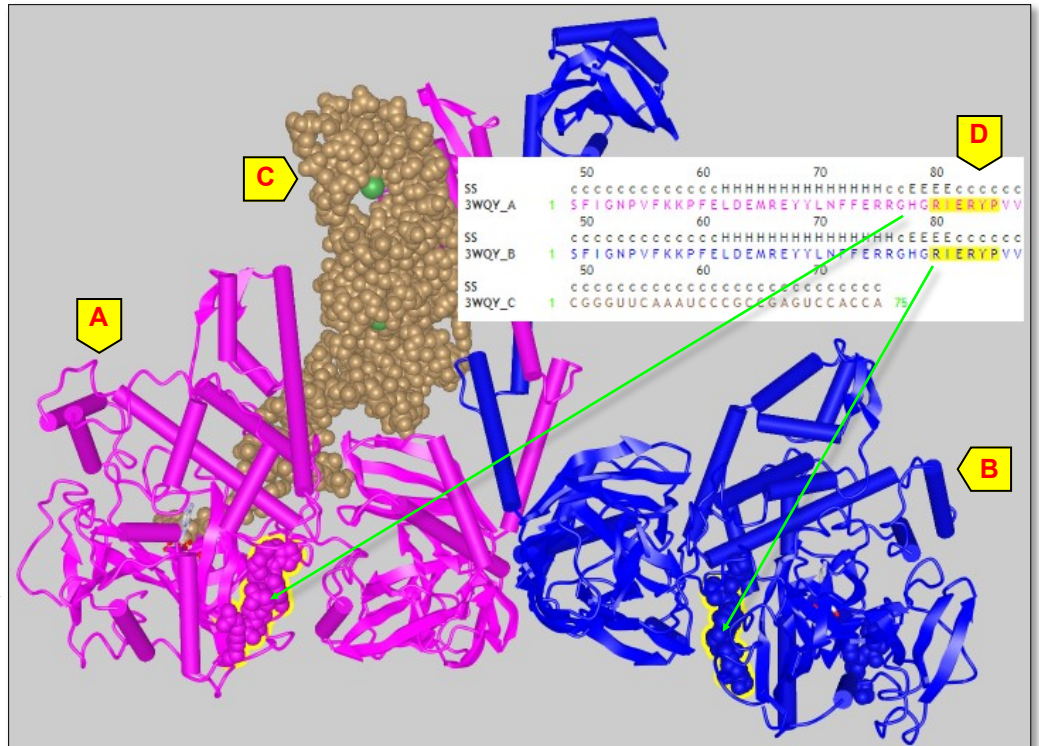
## Case 5 (cont.)

### Steps (cont)

- To view the solved structure, go back to the [3WQY\\_A](#) protein record. Click on the thumbnail graphic of the structure on the right side of the page. Four structure records are shown. Click on the “**View in iCn3D**” link for PDB ID: 3WQY (the second record).
- The structure contains both the **\_A (A)** and **\_B (B)** chains, plus a tRNA(ala) **(C)**. Locate the motif 1 residues in the sequence by using the browser’s “find in page” function to search for RIERY. You find this motif at residues 80-84. Click and drag over RIERY to highlight those residues in yellow on both the sequence and in the viewer **(D)**.

### Interpretation

- You can examine the other features in the 3WQY sequence to confirm their similarity to the members of the conserved domain, and do the same analysis with the other domains. This type of analysis increases confidence in the proposed annotation.
- Starting with a blastx search against the pdb database is one of several approaches that lead to conserved domains and structure records for your annotated proteins. You can also use the records for the proteins found in a blastx search against nr or the landmark database, such as WP\_010877290, an alanine-tRNA ligase identified in Case 4.
- If an ORF finding tool is in your workflow, that also will identify potential coding regions. Our web-based ORFfinder tool accepts nucleotide sequences up to 50 KB, and allows you to directly submit ORFs to the blastp service, <https://www.ncbi.nlm.nih.gov/orffinder>. A standalone version of ORFfinder is also available for Linux, <https://ftp.ncbi.nlm.nih.gov/genomes/TOOLS/ORFfinder/linux-i64/>.



## Appendix

Please address questions on the above example cases to the NCBI blast-help group:

[blast-help@ncbi.nlm.nih.gov](mailto:blast-help@ncbi.nlm.nih.gov)

For questions and feedback on subjects not related to BLAST, email:

[info@ncbi.nlm.nih.gov](mailto:info@ncbi.nlm.nih.gov)

You can check the NCBI Learn page for links to help documents, information on webinars, and workshops:

<https://www.ncbi.nlm.nih.gov/learn/>

The “Tutorials: BLAST” video playlist from NCBI’s YouTube channel can be found at:

<https://www.youtube.com/playlist?list=PLH-TjWpFfWrtjzMCivUe-YbrlleFQIKMq>

Factsheets on popular resources and common tools are available at:

<https://ftp.ncbi.nlm.nih.gov/pub/factsheets/README.html>